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(54) Title: METHODS OF PRODUCING AND SCREENING COMPLEX CHEMICAL LIBRARIES

(57) Abstract

Methods for producing complex mixtures of compounds are provided, where in the first or subsequent stage, a plurality of vessels are employed. In each vessel is present a polyfunctional core molecule, where the functionalities have similar reactivities, and adduct molecules which react at a similar rate under the conditions of the reaction. Each vessel has a set of overlapping but different adduct molecules, so as to provide a diverse set of products. The products are then screened for activity, where a particular reaction mixture is then analyzed by repeating the process of dividing the adducts into overlapping but different sets in different vessels and then screening the vessels for activity. In this manner, a single compound or group of compounds which can be analyzed and screened can be obtained, so as to define the compound(s) which demonstrates the designated activity.

METHODS OF PRODUCING AND SCREENING
COMPLEX CHEMICAL LIBRARIES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of application Serial No. 08/151,727, filed November 12, 1993.

INTRODUCTION

Technical Field

The field of this invention concerns the preparation of complex mixtures of organic compounds, identification of activity, and identification of individual compounds having the activity.

Background

Synthetic organic compounds play an extraordinary role in the well being of modern society. Synthetic organic compounds find application as therapeutics, as pesticides, as additives in a wide variety of context, as dyes, as well as in many other environments. Over the years numerous synthetic organic compounds have been produced, particularly those compounds, where there has been some *ab initio* reason for believing that the compound would have an activity of interest. This belief could be predicated upon an activity of a natural occurring compound, some insight into the nature of the target, or the like. The breadth of compounds that have been produced is quite staggering in number, but the variety of opportunities in producing organic compounds, due to the versatility of carbon and the numerous groups that can be

used to functionalize carbon atoms, leaves a vast
unexplored area.

5   While increasing sophistication in instrumentation
and knowledge has expanded the ability to produce novel
synthetic organic compounds, it has also greatly increased
the cost. Thus, producing individual organic compounds to
develop a class of compounds for screening has become an
extremely expensive exercise, particularly where there is
no assurance that any of the candidate compounds of the
10   class will be found to be active. There has, therefore,
been substantial activity in developing alternative
protocols for producing candidate compounds for
investigation as to their physiological activity, physical
characteristics, and utility.

15   One approach has been commonly called "combinatorial
chemistry." This approach employs numerous reagents in a
series of steps, which allows for the simultaneous
production of large numbers of compounds.

Combinatorial chemistry is a very powerful strategy.
20   Combinatorial libraries make large additions to the
chemical databases, typically used in the pharmaceutical
industry; combinatorial chemistry effectively combines all
the laborious tasks in traditional drug discovery, e.g.
collection and testing of natural product samples,
25   isolation and purification of active ingredients,
structure determination and synthesis, into one rapid
series of steps; since combinatorial chemistry creates
such a large pool of potential active compounds, the
candidate compounds are often more specific than those
30   found in nature or synthesized by traditional means; small
organic compounds make up the vast majority of drugs and
other physiologically active compounds, and large numbers
of small organic molecules can be made over short periods
of time.

35   The methodology requires an accessible means for
determining the nature of a product which is active as to
a particular property or characteristic. The requirement

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for identification of a product which is found to be
active has severely limited the approaches used to produce
large libraries.

One approach has been the preparation of oligomers,
5   such as oligopeptides and oligonucleotides. These
approaches have involved a wide variety of methods for
producing the oligomers and determining their structure,
as well as screening the oligomers for activity. However,
despite the very substantial efforts involved and the
10 numerous protocols for their preparation and screening,
there have been no reported significant successes in
therapeutically useful drug development. One of the
problems is that the oligomers are relatively flexible and
it is generally believed that fairly rigid molecules will
15 be required for binding, where a target molecule is
involved, to achieve the end purpose. The linear
biopolymers, such as oligonucleotides, peptides and
peptidomimetics suffer from poor oral bioavailability,
being too large to be absorbed and too readily degraded
20 physiologically. Efforts have therefore been directed to
synthetic techniques for synthesizing small, organic
molecules. Bunin and Ellman, *J. Am. Chem. Soc.* 114, 10997
(1992), reported a combinatorial approach to the synthesis
of diazepams. By using a few steps and a relatively
25 limited repertoire, Ellman was able to synthesize a
library of diazepam compounds. This approach is fairly
restricted in the number and type of compounds which can
be produced, since the products must be produced one at a
time.
30   Recently, an approach to combinatorial libraries was
reported by Stills' group involving particles, where
reactions are carried out in separate vessels on particles
using different reagents in the different vessels, and the
particles in the vessels tagged with chemical compounds.
35 Ohlmeyer et al., *Proc. Natl. Acad. Sci. USA* 90, 1464-1468
(1993). After each series of reactions, all of the
particles are combined together and then separated into

separate vessels for the next series of reactions and
tagging. After screening the compounds which are
produced, those particles which carry an active compound,
can then be analyzed as to the synthetic sequence which
5   has been employed with that particle by reading the tags.
        Because of the great opportunities that combinatorial
chemistry affords, it is important that new approaches be
devised for the preparation of libraries, which can afford
advantages over the presently existing protocols.

10   <u>Relevant Literature</u>
        Review articles include Baum, Chemical and
Engineering News, 72, 20-26, (1994), Baum, *ibid* 71, 33-34
(1992), and Amato, *Science* 257, 330-331 (1992). Patents
of interest include U.S. patent nos. 5,182,366; 5,270,170;
15   and 5,252,296. PCT applications of interest include
WO94/06017; WO94/04558; WO94/02515; WO93/20242;
WO93/19205; WO93/06121; WO92/00091; and WO91/17283.
        Other references of interest include Fodor et al.,
Science 251, 767 (1991); Lam et al., *Nature* 354, 82
20   (1991); Houghten et al., *Nature* 354, 84 (1991); Blake and
Davis, *Bioconjugate Chem.* 3, 510 (1992); Schultz et al.,
Science 261, 1303 (1993); Brenner and Lerner, *Proc. Natl.
Acad. Sci. USA* 89, 5381 (1992); and Janda et al., *J. Am.
Chem. Soc.* 115, 9812 (1993).

25   SUMMARY OF THE INVENTION
        A method for producing a library of related molecules
is provided employing a polyfunctional core molecule and a
plurality of adduct molecules capable of reacting with the
core molecule functionalities. A plurality of non-
30   overlapping adduct subset units are defined to be used to
define the adduct reaction set. As a first or
intermediate step, in each of a series of vessels one of
overlapping different sets of adduct molecules comprised
of less than the total number of subset units are added to
35   the core molecules under reaction conditions, where

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usually the reaction rate in each vessel of each of the
adduct molecules is substantially normalized. The content
of each vessel is then screened for activity as to one or
more utilities. For each vessel which shows activity, a
5   second series of reactions may be carried out using the
core molecules and a set of adduct molecules having a
reduced number per vessel of adduct molecules coming from
within the set which was active. This set may be selected
to ensure that all the molecules produced in the active
10   set are produced or, by comparing the results of the
overlapping sets, one or more of the adduct molecules may
be excluded from the set as not being involved in an
active product. A screening program is provided to
rapidly narrow a large number of candidate molecules to a
15   relatively small number of candidate molecules, which may
then be characterized by physical or other means as to the
identity of the active compound(s).

DESCRIPTION OF SPECIFIC EMBODIMENTS

In accordance with the subject invention, complex
20   mixtures, which are non-polymeric synthetic organic
molecules are produced, where a plurality of vessels are
employed, each vessel containing the same polyfunctional
core molecule. The polyfunctional core molecule may have
the same functionalities [poly(mono)functional] or
25   different functionalities having similar reactivity to the
adducts [poly(multi)functional]. To each vessel is added
a set of reactants ("adducts") capable of reacting with
the functionalities present on the core molecule. Non-
overlapping subsets of adducts are chosen to define
30   overlapping different sets of adducts. The sets are
selected to provide all possible combinations of products
resulting from reactions with the members of all of the
sets. The reaction is allowed to proceed in each of the
vessels to at least substantial completion. The
35   conditions of the reaction, including the concentrations
of each of the adducts, are selected so as to

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substantially normalize the rate of reaction of each of
the adducts with the core molecule functionality and to
minimize undesired side reactions. After completion of
the reaction, all or aliquots of a reaction mixture from a
5   single vessel may then be screened for activity as to a
particular purpose. Any activity may be considered or a
minimal threshold activity may be selected for a
particular mixture as defining activity.

As to those vessels where the activity exceeds the
10 selected threshold activity, a second series of reactions
may be carried out. Once again, a plurality of vessels is
employed, where the core molecule is present in each
vessel, and individual sets of adducts are added to each
vessel, where the sets have fewer adducts than the
15 previous set, and may provide less than all of the
compounds which were produced in the previous reaction
vessel up to all of the compounds. Where less than all of
the compounds will be produced, this may be as a result of
analysis of activity of the various mixtures,
20 demonstrating that certain combinations will not be active
or allowing for the absence of certain combinations, where
lack of activity in a vessel would indicate that the
compound.(s) which was not produced provided the activity
in the previous vessel. This process may be repeated as
25 many times as necessary to obtain a single product or a
mixture of products which can be analyzed, so as to
identify the individual products in the mixture, where the
individual synthesis of all the products is not onerous.

The number of adduct molecules will usually be at
30 least 6, more usually at least 8, generally at least about
12, usually not exceeding 200, more usually not exceeding
100.

The step or stage comprising a plurality of adducts,
where sets of adducts are used in different reaction
35 vessels with the same core molecules, may be ·the first
step or an intermediate step. While one may carry out a
single reaction in a single vessel with a set of all the

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adducts, and then screen the entire mixture for activity, generally this will not be done. More usually, one will begin as a first step using a plurality of vessels, at least two, more usually at least three, preferably at

5   least about five, and usually not more than 300, more usually not more than 200, and preferably not more than 100. The number of vessels will be dictated by the number of functionalities on the core molecule, the number of adducts, the desired complexity of the mixtures, the

10 sensitivity of the assay(s) for screening activity, the amounts of products needed, and the like.

Usually, the system will provide for the preparation of at least about 100 compounds, more usually at least about 200 compounds, preferably at least about 500

15 compounds, frequently at least about 1,000 compounds, usually fewer than about 5×10^6 compounds, more usually fewer than about 1×10^6 compounds. While for the most part it may be assumed that the primary products will be the per-substituted core molecule, one may anticipate that

20 in many situations, there will be small but identifiable amounts of core molecules which have reacted at fewer than the total number of functionalities present.

In carrying out the subject invention, a "primary" list of adduct molecules is made. This list defines the

25 variety of products which may be achieved in relation to the core molecule. As a convenient tool, this list may be divided into non-overlapping subset units, where the number of units is equal to or greater than the number of reactive functionalities on the core molecule. These units

30 are then combined to provide different overlapping sets of adducts for reaction in different vessels, so that the total number of possible products from the adducts is obtained. Each vessel will have a different mixture of products, having a proportion of products which overlap

35 the products in other vessels. While still obtaining the total number of products available from the number of adducts used, screening is improved in that fewer than the

total number of products is present in each vessel, allowing for fewer iterative steps to define active compounds, with less chance for interactions between compounds confusing the results of the assays. As one
5 finds activity in a particular vessel, one can then repeat the process, using the list of adduct molecules used in that particular vessel to define the total number of adduct molecules which are to be distributed among the plurality of vessels in accordance with the previously
10 described strategy in the next step.

The total number of products produced by the subject method is indicated by the following:

$$R^i-X + Z-(Y)_v \longrightarrow Z(-W-R^i)_v$$
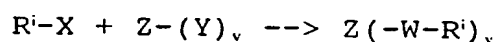
where i=1 to n.

15 The equation indicates that there are "n" different adduct molecules, (R^i) where i=1 to n, which will be reacted with a core molecule having "v" reactive functionalities ("Y"). The products all have the same core moiety "Z," but have random covalently bonded adduct
20 moieties, where the R groups attached to the core moiety may be the same or different. X is a reactive functionality, which reacts with Y to form a covalent bond. W is the linking group formed from the reaction of X and Y.

25 Illustrative of the reaction situation would be a core molecule with four reactive functionalities. When the core molecule has no axis or plane of symmetry, each of the reactive centers is unique and the number of possible products is given by "n^v". If the reaction
30 utilizes 50 adduct reagents, then the number of expected products is $50^4 = 6,250,000$.

The screening strategy should efficiently narrow the number of adduct molecules used at each step, while ensuring that each of the possible products produced by
35 the original adduct set is formed in at least one of the subsets. To accomplish this, the primary adduct set, S_i, is divided into "k" non-overlapping partial subsets, p_i,

with equal numbers of adducts ($\geq$ 1 adduct per subset),
where a subscript "i" denotes an integer of from 1 to k (k
being greater than the number of reactive functionalities
on the core molecule). Secondary subsets $s_{2,j}$, are then
5   prepared by making each possible combination or union set
of "v" partial subsets (v is the number of reactive
functionalities on the core molecule and the subscript j
is an integer from 1 to m, where m is the number of
subsets). The secondary subsets are then used to
10 synthesize secondary product mixtures for screening.
    For example, if a trifunctional asymmetrical core
molecule is reacted with a primary adduct set of 12
different adduct molecules, the following methodology can
then be used to identify an active compound from among the
15 12^3 = 1728 products. The original adduct set of 12 adduct
molecules (termed A,B,C,D,E,F,G,H,I,J,K,L) is divided into
a number of non-overlapping partial subset units. Here
the number of partial subset units, k, is selected to be
greater than the number of reactive functionalities on the
20 core molecule. In this example using a core molecule with
three reactive functionalities, the adduct list is divided
into k=4 partial subsets p_i.

$$s_1 = \{A, B, C, D, E, F, G, H, I, J, K, L\}$$
$$p_1 = \{A, B, C\}$$
25
$$p_2 = \{D, E, F\}$$
$$p_3 = \{G, H, I\}$$
$$p_4 = \{J, K, L\}$$

In this example, the number of different union sets, $s_{2,j}$,
that can be made by combining 3 of the 4 partial subsets
30 is m = 4. In general, the value for m can be determined
from the equation m = (k choose v) = $k!/(v! \times (k-v)!)$,
where k is the number of non-overlapping partial subsets
and v is the number of reactive centers on the core
molecule. Here, m = (4 choose 3) = 4!(3! x 1!) = 4. The
35 4 secondary subsets, $s_{2,j}$, are:

$$s_{2,1} = p_1 \cup p_2 \cup p_3 = \{A, B, C, D, E, F, G, H, I\}$$
$$s_{2,2} = p_1 \cup p_2 \cup p_4 = \{A, B, C, D, E, F, J, K, L\}$$

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$$s_{2,3} = p_1 \cup p_3 \cup p_4 = \{A, B, C, G, H, I, J, K, L\}$$
$$s_{2,4} = p_2 \cup p_3 \cup p_4 = \{D, E, F, G, H, I, J, K, L\}$$

In this example, the original set of 12 adduct types has been reduced to four subsets of 9 adduct types. Most

5 importantly, all combinations of three adducts that can be chosen from the original primary set, s_1, are present in at least one of the secondary subsets, $s_{2,j}$. Accordingly, four adduct reagent mixtures would be prepared and separately reacted with the core molecule to produce four

10 different product mixtures for screening. Each secondary product mixture would contain $9^3 = 729$ products, compared to the primary product mixture (using all twelve adducts) which would contain $12^3 = 1728$ products. After screening the four mixtures and identifying the most active mixture,

15 a series of 4 smaller tertiary adduct subsets could be defined to continue the process.

To identify an active compound in a product mixture using fewer iterations, a higher value of k is favored. The above example can be repeated for k = 6 and v = 3.

20
$$s_1 = \{A, B, C, D, E, F, G, H, I, J, K, L\}$$
$$p_1 = \{A, B\}$$
$$p_2 = \{C, D\}$$
$$p_3 = \{E, F\}$$
$$p_4 = \{G, H\}$$

25
$$p_5 = \{I, J\}$$
$$p_6 = \{K, L\}$$

The number of different union sets, $s_{2,j}$, that can be made by combining 3 of the 6 partial subsets is m = (6 choose 3) = 20. The 20 secondary subsets, $s_{2,j}$, are:

30
$$s_{2,1} = p_1 \cup p_2 \cup p_3 = \{A, B, C, D, E, F\}$$
$$s_{2,2} = p_1 \cup p_2 \cup p_4 = \{A, B, C, D, G, H\}$$
$$s_{2,3} = p_1 \cup p_2 \cup p_5 = \{A, B, C, D, I, J\}$$
$$s_{2,4} = p_1 \cup p_2 \cup p_6 = \{A, B, C, D, K, L\}$$
$$s_{2,5} = p_1 \cup p_3 \cup p_4 = \{A, B, E, F, G, H\}$$

35
$$s_{2,6} = p_1 \cup p_3 \cup p_5 = \{A, B, E, F, I, J\}$$
$$s_{2,7} = p_1 \cup p_3 \cup p_6 = \{A, B, E, F, K, L\}$$

$$S_{2,8} = p_1 \cup p_4 \cup p_5 = \{A, B, G, H, I, J\}$$
$$S_{2,9} = p_1 \cup p_4 \cup p_6 = \{A, B, G, H, K, L\}$$
$$S_{2,10} = p_1 \cup p_5 \cup p_6 = \{A, B, I, J, K, L\}$$
$$S_{2,11} = p_2 \cup p_3 \cup p_4 = \{C, D, E, F, G, H\}$$
$$S_{2,12} = p_2 \cup p_3 \cup p_5 = \{C, D, E, F, I, J\}$$
$$S_{2,13} = p_2 \cup p_3 \cup p_6 = \{C, D, E, F, K, L\}$$
$$S_{2,14} = p_2 \cup p_4 \cup p_5 = \{C, D, G, H, I, J\}$$
$$S_{2,15} = p_2 \cup p_4 \cup p_6 = \{C, D, G, H, K, L\}$$
$$S_{2,16} = p_2 \cup p_5 \cup p_6 = \{C, D, I, J, K, L\}$$
$$S_{2,17} = p_3 \cup p_4 \cup p_5 = \{E, F, G, H, I, J\}$$
$$S_{2,18} = p_3 \cup p_4 \cup p_6 = \{E, F, G, H, K, L\}$$
$$S_{2,19} = p_3 \cup p_5 \cup p_6 = \{E, F, I, J, K, L\}$$
$$S_{2,20} = p_4 \cup p_5 \cup p_6 = \{G, H, I, J, K, L\}$$

Here, the original set of 12 adduct types has been reduced
to 20 subsets of 6 adduct types. From these secondary
subset definitions, 20 adduct reagent mixtures would be
prepared and separately reacted with the core molecule to
produce 20 different product mixtures for screening. Each
secondary product mixture would contain $6^3 = 216$ products,
compared to the primary product mixture (using all twelve
adducts) which would contain $12^3 = 1728$ products. After
screening the 20 mixtures and identifying the most active
mixture, a series of 20 smaller tertiary adduct subsets
could be defined to continue the process.

In this example, where v=3 and k=6, one iteration of
the screening process decreases the number of adducts per
subset by the factor 1/2, and the number of products per
mixture by 1/8. These factors would narrow an adduct list
in significantly fewer steps. However, a larger number of
product mixtures must be screened to achieve this

narrowing of the adduct list in fewer iterations. When
the screening assays are automated and a 96-well
microliter plate is employed, it is efficient to produce
as many as 96 different product mixtures or more at each
5 step.

The Table below provides some preferred values for
the number of partial subsets, the number of secondary
product mixtures and the reduction in the number of adduct
types for each iteration when core molecules with two,
10 three, four and five reactive functionalities are used.

Table

Reactive functionalities (v)	Partial Subsets (k)	Product Mixtures (m)	Reduction Factor (v/k)
2	4	6	1/2
2	6	15	1/3
2	8	28	1/4
2	10	45	1/5
3	6	20	1/2
3	9	84	1/3
3	12	220	1/4
4	8	70	1/2
5	10	252	1/2

The reduction factor (v/k) is the amount by which the
25 number of adduct types are reduced after each step of
screening. Preferred reduction factors are those having

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reciprocals which are integers. Such reduction factors
can be repeatedly and evenly applied to initial adduct
sets. Particularly preferred combinations of v and k are
the combination v=3, k=9; v=3, k=6; and the combination

5   v=4, k=8. For these particularly preferred combinations,
the number of product mixtures can be easily contained in
a 96-well microtiter plate.

The vessels employed may be any convenient vessels,
which provide for the desired volume. The core molecule

10 will normally be present in an amount of from about 0.1
millimole to 10 millimole. Normally, each adduct molecule
will be present in at least about 0.5 millimole, usually
at least about 2 millimoles and can be up to 100
millimoles or more in each mixture, usually not exceeding

15 about 50 millimoles, more usually not exceeding about 10
millimole in each mixture. As already indicated, the
amount of individual compounds which will be produced will
be related to the minimal activity of interest,
sensitivity of the assay, ease and economics of synthesis,

20 and the like. Reaction volumes will usually be at least
about 1 ml, more usually at least about 5 ml, and may be
0.5 L or more, usually not exceeding about 100 ml. The
concentration of the core molecule will generally be in
the range of about 1 mM to 10M, more usually about 10 mM

25 to 1 M. Generally, the core molecule will be in a mol
ratio of about 0.02 to 1 to each of the adduct molecules,
depending on the relative rate of each of the adduct
molecules, the desired rate of reaction, the volume of the

reaction mixture, and the like. Solvents will be chosen

in accordance with the nature of the reaction, where the

solvents may be polar or non-polar organic or inorganic,

combinations thereof, single or mixtures of solvents, and

5   the like. Illustrative solvents include dimethylformamide,

DMSO, ethyl ether, propanol, acetonitrile, toluene,

anisole, acetone, and the like. The core molecule and

adducts will be combined, either neat or in the presence

of a solvent, to carry out the reaction to form the

10 mixture. Temperatures will depend upon the particular

reaction, generally ranging from about -10 to 100°C. The

core molecule, when present initially, will generally be

at a concentration in the range of about 1 mM to 1 M, more

usually in the range of about 10 mM to 100 mM. The

15 concentrations of the reactants may vary depending upon

the manner and order of addition.

As indicated previously, it will usually be desirable

to normalize the reactivity of the individual adducts with

the functionalities of the core molecule. By

20 normalization it is intended that the difference in rates

between any two adducts be not greater than twelve-fold,

preferably not greater than ten-fold, and more preferably

not greater than about five-fold. Therefore, the

concentrations will be selected, so that there will be a

25 reasonable opportunity to prepare all possible per-

substituted compounds, where the difference in rate will

not result in substantial exhaustion of one or more

adducts during a period where there has been little or no

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reaction of one or more other adducts. The relative rates
of reaction of the individual adducts can be readily
determined by setting up a pairwise experiment in which
two adducts are reacted with the core molecule. The

5   relative rates of reaction of the adduct of interest is
directly related to the factor which affords equimolar
yields of the two aforementioned adducts. In this way,
one can readily define the various ratios to be used in
each of the vessels for production of a particular

10 composition mixture. Desirably the yield range should not
be greater than about 2 to 10, preferably not greater than
about 2 to 5.

Another method for normalization is to choose one
adduct reagent to be a "normalization standard," and for

15 each of the other adduct reagents, prepare an equimolar
binary mixture of that reagent and the normalization
standard. Each binary adduct mixture is then reacted with
the core molecule and the "relative reactivity" of each
adduct relative to the normalization standard is readily

20 determined from the product yields.

Various orders of addition may be employed, where the
core molecule is added to the preformed mixture of
adducts, the core molecule and adducts are added
substantially simultaneously or the adducts are added to

25 the core molecule at the same or different rates and at
the same or different concentrations, to substantially
maintain the normalized rate of reaction of the various
adducts.

The adduct molecules will usually be employed in greater than stoichiometric amounts, (the number of moles being equivalent to the proportion of reactive functionalities which the adduct will react with based on

5   the number of adduct molecules and the number of different adduct reagents), generally in at least 2 fold molar excess, usually at least about 5 fold molar excess, and may be about 75 fold molar excess or more, generally less than about 50 fold molar excess. By having adduct

10 molecules with similar rates of reaction with the reactive functionalities, the amounts of each of the adduct molecules will be relatively similar.

The core molecule may be free in solution or be bound to a solid support, where the solid support may be resins,

15 e.g. Merrifield or modified versions thereof, silica-based support, e.g. aminopropyl silica (ref. J. Am. Chem. Soc. 116:1135 [1994]), solid surfaces such as magnetic particles, porous glass beads, polyethylene pins, etc. One of the advantages of the subject methodology is to

20 have all of the reactants in solution, so that homogeneous reactions in solution will be preferred. Where particles are employed, one may join the core molecule to the particle by a functionality which is readily cleavable, while retaining the various adducts. A wide variety of

25 molecules can be cleaved preferentially, e.g., thiophenyl ethers or silyl compounds which may be cleaved by mercuric trifluoroacetate or fluorides (tetrabutylammonium

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fluoride), nitrobenzyl ethers, which can be cleaved by photolysis, *etc.*

For the most part, the reaction will be carried out with agitation, so as to ensure a substantially uniform

5 mixture. Agitation can be achieved by rocking, stirring, shaking, or other convenient means.

A wide variety of addition or condensation reactions may be employed to produce the products. Because of the versatility of the system, the adducts may have the same

10 or different functionality, so long as the required normalization can be achieved. The functionalities on the adduct molecules may include such reactive groups as halogen, usually other than fluorine, where the halogen may be used in a Grignard (RMgX) or Reformatsky (RZnX)

15 reaction, oxy, oxo, ketone or aldehyde, carboxylic acids and derivatives thereof such as acid halides, esters and anhydrides, amino, hydrazino, ylides, cyanate, isocyanate, isothiocyanate, olefinic or acetylenic unsaturation, small alicyclic or heterocyclic rings, such as cyclopropyl,

20 oxiranyl, and aziridinyl, phosphoric acids, such as phosphates, phosphinates, phosphinamides, phosphoryl halides, etc., sulfuric acid derivatives, including sulfonates, sulfates, and sulfinates, thiol, disulfide, nitriles, carbamates, thiocarbamates, imidate esters, and

25 the like. Functionalities which may be produced include esters and amides, from both organic and inorganic acids, ethers, both oxy and thio, imines, hydrazones, chiral epoxides from the asymmetric oxidation of alkenes with an

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enantioselective catalyst (J. Am. Chem. Soc. 116:6937-8
[1994]), etc.

The adduct molecules will be selected to
substantially reduce or eliminate unimolecular or
5   bimolecular side reactions, such as elimination,
condensation, addition, and the like. For the most part,
with active halides, elimination will be the major side
reaction under basic or acidic conditions. Therefore,
substitution at the α- or β-position will usually be
10 limited to the presence of at least one hydrogen atom for
aliphatic molecules and heterofunctionalities or other
functionalities, which might aid elimination will be
avoided at these positions. Ester and carbonyl groups
will usually be avoided in basic media. The reactions of
15 the common functionalities are well known and obvious side
reactions can be avoided.

The core molecule may be aliphatic, alicyclic,
aromatic or heterocyclic, where the heteroatoms will
usually be nitrogen, oxygen and sulfur, although other
20 heteroatoms, such as phosphorous, metal atoms, boron, etc.
may be present. In some instances, metallocenes may serve
as the core. Usually, there will be at least two active
functionalities, more usually at least three active
functionalities, and not more than about ten active
25 functionalities, more usually not more than about six
active functionalities, desirably 3 to 5, particularly 3
to 4. The active functionalities may be symmetrically
situated, so as to have the same reactivity or may be

asymmetrically situated, so as to have differing activity.
Compounds of interest include sugars, such as mono- and
disaccharides, polyfunctionalized aromatic compounds,
where the aromatic core may be carbocyclic or

5 heterocyclic, such as benzene, naphthalene, biphenyl,
pyridine, etc., may be alicyclic where the rings may be mono-
or bicyclic or higher order, such as cyclohexane,
cyclooctane, adamantane, bicyclo-heptane; acyclic organic
compounds, such as ethylenediamine tetracetic acid,

10 nitrolotriacetic acid, tris-hydroxyethylamine, 2,2,2-tri-
thiolmethyl-1-methoxyethane, etc. To minimize steric
interactions during the reaction and in the product,
active functionalities on the core molecule will
preferably be separated by a sufficient distance to

15 minimize steric hindrance or other interactions which
would substantially change the reactivity of an active
functionality with the adduct reagents, either before the
reaction of one of the active functionalities or after
reaction of one of the reactive functionalities with an

20 adduct reagent. While not universally true, two active
functionalities will be separated by at least about 3
atoms and be bonded to an atom, usually carbon, which when
saturated will usually be bonded to at least one hydrogen
atom. Steric effects may be diagnosed by running

25 reactions with a core molecule and a single adduct type
and verifying that the main product is per-substituted.

    While the core molecules may be symmetrical or
asymmetrical, the symmetrical core molecules yield fewer

products, but the products are easier to separate and identify, as compared to asymmetrical molecules. For example, comparing 1,2,6-hexane and 1,3,5-trihydroxybenzene, the latter compound has both a C_3 and C_2 axis of symmetry. The addition of five adduct molecules to the asymmetrical 1,2,6-trihydroxyhexane should in principle lead to $5^3=125$ distinct, trisubstituted products. The trigonal symmetry in 1,3,5-trihydroxybenzene reduces this to 35 as the number of distinct trisubstituted products is given by the formula, $(n^3 + 3n^2 + 2n)/6$.

In some situations, adduct molecules may be difunctional, where the adduct molecule can react with two functionalities on the core molecule to form a ring. In these situations, the core molecule will usually have two vicinal functionalities or be situated in spatial proximity, e.g. 1,8-disubstituted naphthalene, where the resulting rings will have from about 5 to 7 annular members. Illustrative situations include dihalides with glycols, activated dicarboxylic acids with diamines, diisocyanates with diamines, etc.

The linking group formed by the reaction between the core molecule functionality and the adduct functionality may take many forms, including carbon-carbon, carbon-oxygen, carbon-nitrogen and carbon-sulfur bonds. The reactions may be addition, substitution, elimination, condensation, free radical, or other reaction, as appropriate. Reactions which may be involved include

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esterification, amidification, etherification, addition to
unsaturation, Claisen condensation, metal catalyzed
coupling reactions, asymmetric epoxidation or
hydroxylation, *etc*. For carbon-carbon bond formation, one

5   may use the Grignard reagent with an oxo or non-oxo-
carbonyl functionality or the oxo functionality or an
active halide with an organolithium compound. Suitable
reactions may be found in March, "*Advanced Organic
Chemistry*", 4th ed., Wiley-Interscience, NY, 1992,

10 incorporated herein by reference. In particular, pages
417-424 relate to amide formation, page 903 to urea
formation, pages 896-898 to imine formation, pages 386-387
to ether formation and pages 920-930 to Grignard
reactions.

15   Reaction conditions will be optimized to provide for
high yields of completely reacted core molecules, where
all of the reactive functionalities have reacted with the
adduct molecules. Conditions can be chosen to drive the
reaction to completion, such as large excesses of the

20 adduct molecules, elevated temperatures and pressures,
long reaction times, where feasible, segregation of
product from the main reaction mixture, catalysts, or the
like. Segregation can be achieved where the final product
has a different solubility from the core molecule and

25 intermediate products, so that the final product may be
taken up in a selective solvent. Functionalization of
certain functionalities to enhance reactivity may be
employed. With carboxylic acids, active esters may be

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formed, e.g. pentafluorophenyl or o-nitrophenyl esters, or carbodiimides may be added. Lithium derivatives may be prepared from amides or amines. Metal ions may be added to enhance substitution reactions.

5   The creation of stereogenic centers via asymmetric induction with chiral catalysts may also be used. Thus, chiral secondary alcohols may be prepared from the enantioselective reduction of ketones, while highly enantioselective formation of epoxides can result from the

10   oxidation of simple olefins in the presence of a chiral manganese catalyst.

At the end of the reaction, the reaction mixture may be subjected to a variety of treatments. Any particular treatment will depend upon the nature of the products, the

15   purpose for which the product is to be screened, the solvents used, and the like. Any remaining adduct molecules may be removed by any convenient means. In some situations where an organic solvent is used, the solvent may be removed and the resulting product dispersed in an

20   aqueous medium, an aqueous organic medium, or organic medium e.g., DMSO, DMF, ethanol, etc. Where the product has been produced bound to a surface, the product may be released from the surface as described previously, based upon the labile linking group. In some instances, small

25   aliquots may be taken and analyzed in a gas chromatograph, GC-MS, capillary electrophoresis, or the like, so as to obtain some indication of the number of different compounds which have been produced. In some situations,

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protective groups may have been employed with

functionalities present on the adducts. The protective

groups may be removed in accordance with conventional

ways, depending upon the nature of the protective group,

5   as well as the nature of the products. For example, amino

groups may be protected with FMOC, where the FMOC groups

may be removed with piperidine in DMF.

Various techniques may be employed to separate the

products from the excess adduct molecules. Such

10   techniques as distillation, chromatography, e.g. ion

exchange chromatography, solvent extraction, base or acid

extraction, or the like, may be employed in accordance

with the nature of the products and adducts. If desired,

the product mixture may be fractionated in accordance with

15   a particular characteristic, to give more information

about the nature of an active product.

After completion of the post processing of the

product mixture, the product mixture is then screened for

activity. The product mixture may be screened for one or

20   more activities, depending upon the nature of the product.

One screening may be for binding affinity. Thus, the

subject compositions may be screened for their ability to

bind specifically with reasonable specificity to a target

molecule. Thus, the subject compositions may be screened

25   as enzyme inhibitors, as molecules for directing another

molecule to a specific site, e.g. a physiological site,

such as a blood protein, a surface membrane protein,

specific organs, plant parts, pathogens, and the like. In

some instances, the subject compounds will be evaluated

for activity, as cytotoxic agents, for inducing a signal

across a surface membrane protein, as a competitor to a

natural ligand, or the like. The subject compounds may

5   also find use in diagnostics, where they may serve as

reagents for competition with an analyte, binding to a

ligand of interest, as labels, enzyme substrates, and the

like. The subject compounds may also be evaluated for

binding to nucleic acids, sugars, etc.

10   As illustrative of screening, for determining binding

affinity, a number of different techniques may be

employed. One may pass the mixture through a column in

which the target molecule is bound to a solid support.

From such a column, weakly binding or non-binding

15   compounds will be eluted. After washing the column, one

may then use an isocratic or gradient solvent with

increasing polarity or increasing ionic strength, so as to

obtain different fractions of compounds which bind,

depending upon the effect of the solvent on the binding

20   affinity. Where a fraction has one or few compounds,

usually fewer than five, one may be able to analyze the

compounds in the fraction to determine their structure.

Even if a complete structure cannot be determined,

information may be obtained so as to reduce the number of

25   adducts required at the next stage.

One may do a gross determination by combining the

product mixture with a labeled compound known to bind to

the target. One can then determine whether there is one

24

or more compounds in the product mixture which can
effectively compete with the labeled compound for the
target. Thus, by employing a labeled compound, one can
detect the amount of labeled compound which binds or does

5   not bind in the presence and the absence of the product
mixture. Where an enzyme is the target, one can combine
the enzyme with the product mixture and its substrate and
determine the enzymatic rate. A reduction in rate will
indicate that one or more components of the product

10   mixture are capable of inhibiting, competitively or non-
competitively, the enzyme. Other techniques may also be
employed.

     For biological activity, one may screen the subject
compounds for cytotoxic or stimulating effect. By

15   combining the product mixture with a target unicellular
microorganism in an appropriate medium, one can determine
the rate of proliferation of the microorganism in the
presence or absence of the product mixture. Stimulation
or inhibition may be determined. Other screens may

20   include binding to mammalian cells, where the target may
be associated with homing, transduction of the signal
across the membrane, inhibition of T-cells, binding to MHC
antigens, etc.

     In addition, the subject compounds may be screened

25   for a wide variety of applications as additives, for
fuels, oils, hydraulic fluids, boilers, plastics, food,
cosmetics, etc., as pesticides, stabilizers, antioxidants,
etc.

In each case, the mixture will be employed in
accordance with conventional methods for evaluating a
particular performance.

Where a product mixture has been found to be active,
5 the process is then repeated, where a plurality of vessels
are employed, but the primary set of adduct molecules is
now up to and including the total number used for a
particular vessel in the previous stage, where the product
mixture from the vessel is found to be active. Thus, the
10 number of vessels required and the adducts introduced in
each vessel may be substantially reduced or expanded, as
compared to the first stage, depending upon how few
compounds are to be produced in each vessel. This
iterative process may be repeated as many times as
15 necessary, until one obtains a single compound or a
mixture of compounds which can be individually analyzed.

The following examples are offered by way of
illustration and not by way of limitation.

EXPERIMENTAL

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EXAMPLE 1

Reaction of Resorcinol with Alkyl Halides
General

Unless otherwise indicated all reagents were obtained
25 commercially and used as received without further
purification. Neutral alumina, purchased from Aldrich,
was of Brockmann I activity, ~150 mesh, and used as

obtained. Anhydrous potassium fluoride (KF, Baker grade) was handled under nitrogen, in an aqueous acid-free environment. The GC-MS system used for analysis consisted of (a) a Hewlett-Packard 5890 Series II Plus GC equipped

5   with an HP-5 cross-linked 5% phenyl methyl silicone capillary column (0.25 mm i.d. x 30.0 mm long), interfaced to (b) a Hewlett-Packard 5972A Mass Selective Detector (MSD) equipped with a quadrupole mass filter. The data was processed with the aid of the MS (DOS)

10 Chemstation software. The internal standard used in the gc analysis was an aromatic ether, p-tolyl ether (m/e 198amu).

Preparation of KF/Al$_2$O$_3$

An aqueous solution (200 mL) of KF (1.15 mol, 67g)

15 was slowly poured into a 500 mL round-bottom flask containing neutral Al$_2$O$_3$ (100g). After about 15 min. of agitation to ensure thorough mixing, the resulting mixture was subjected to rotary evaporation at 50-60°C until most of the water had been removed. The now impregnated

20 alumina was next heated in an oil bath kept at ~85 °C under higher vacuum (10^{-3} Torr) for an overnight (~12h) period. The KF/Al$_2$O$_3$ thus prepared can be readily handled in the open atmosphere and is indefinitely stable when stored in a desiccator.

25 Combinatorial Synthesis of a Sample Library of Ether Derivatives of Resorcinol

In a typical preparation, a 50 mL round bottom flask equipped with a magnetic stir bar and addition funnel was charged with resorcinol (1 mmol, 0.11g) dissolved in DMF (25 mL). This was followed by addition of the alkylating

5 ·agent, KF/Al$_2$O$_3$ (10 mmol, 5 mmol/OH equiv., 1.60g). A mixture of three primary alkyl bromides, 1-bromopropane (A, 15.4 mmol), 4-bromobutyl acetate (B, 10.0 mmol), and β-bromophenetole (C, 24.2 mmol), was subsequently added dropwise from the addition funnel to the rapidly stirring

10 dark green-colored suspension. The progress of the reaction mixture was monitored and analyzed by GC-MS until a satisfactory distribution of equimolar yields for the desired products was obtained (48h). The identity of the eluted peaks was established by electron-impact mass

15 spectrometry (EI-MS) showed the molecular ion peak for all compounds of interest, with significantly smaller amounts of mono-O-alkylated derivatives of resorcinol, with no evidence of side reactions leading to unwanted products. Integration of the parent peaks of interest affords the

20 relative yields of all six bis-O-alkylated products (Table 1). At this time, the reaction mixture was evaporated to dryness to remove excess R'CH$_2$Br and other volatiles. The residue was extracted with 1:1(v/v) Et$_2$O:CH$_2$Cl$_2$ (4 x 20 mL) followed by filtration. The filtrate was evaporated to

25 dryness to afford the desired ether derivatives of resorcinol as powders.

TABLE 1

Compound	Ion yield area/10^6	Ion, % total	Total ion yield	M.W. (amu)	Rel. molar yield
AA	0.970	0.094	10.32	194	0.053
AB	3.558	0.094	37.85	266	0.142
AC	1.464	0.089	16.45	272	0.060
BB	3.206	0.094	34.11	338	0.101
BC	2.527	0.086	29.38	344	0.085
CC	0.250	0.065	3.85	350	0.011

$$R = [AB]/[CC] = 13$$

The presence of a two-fold axis of symmetry in resorcinal implies that **AB = BA**, etc. For these products, the relative yields should be twice that for **AA**, **BB**, etc., so that the yield ratio (R) of the most abundant product to the least abundant product should be 2, as compared to the observed ratio of 13. By assuming that the reaction takes place in two alkylation steps, with the rate of each step proportional to: (i) the relative concentration of the alkyl bromide $[B]_{rel}$; and (ii) the relative "reactivity" of the alkyl bromide (k_B). Fitting mathematical expressions corresponding to the yield data in Table 1 are then written as shown in Table 2.

Table 2

Compound	Rel. molor yield, normalized to AA	Fitting expression	Best fit yields
AA	1.00	$[A]^2 k_A^2$	1.00
AB	2.68	$2[A][B]k_A k_B$	2.80

AC	1.13	$2[A][C]k_Ak_C$	1.16
BB	1.91	$[B]^2k_B^2$	1.96
BC	1.60	$2[B][C]k_Bk_C$	1.62
CC	0.21	$[C]^2k_C^2$	0.33

5   By simultaneously using these fitting expressions for each

of the products and solving for the relative constants k_B

and k_C (k_A being set to 1), the values of 1.539 and 0.636

are obtained, respectively. When these values of k_B and

k_C are used in all six fitting expressions, the resulting

10   best fit yields agree remarkably well with the measured

relative yields. According to the method described above,

using [A]:[B]:[C] = 15.4 mmol: 10.0 mmol: 24.2 mmol would

be expected to lead to an equimolar distribution product

yield, with R = 2. In a reaction mixture with the alkyl

15   bromides having the concentrations indicated above, the

relative yields of the six products of interest indicated

an essentially equimolar distribution of product yields (R

= 2.56). This is excellent agreement with theoretical,

where the influence of the first O-alkylation on the

20   second O-alkylation is being ignored.

EXAMPLE 2

Combinatorial Synthesis of a Sample Library of Ether

Derivatives of Phloroglucinol

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In a typical preparation, a 100 mL round bottom flask
was charged with phloroglucinol dihydrate (1 mmol, 0.16g)
dissolved in DMF (25 mL) followed by addition of KF/Al_2O_3
(15 mmol, 2.4g). To the rapidly stirring gray-colored

5   suspension, a mixture of three alkyl bromides: in a first
study A (23 mmol), B (15 mmol) and C (36 mmol); and in a
second study, A (23 mmol, 2.8g), B (18 mmol, 3.5g) and C
(73 mmol, 14.7g) (wherein A, B, and C are as defined in
Example 1), was added dropwise via an addition funnel.

10   After 48h, GC-MS analysis of an aliquot sampled from the
yellow-green reaction mixture showed a satisfactory
distribution of yields for the desired tri-O-alkylated
products. The mixture was evaporated to dryness and
worked up as described above.

15   In the first study CCC could not be detected, which
may have been a result of decomposition during the gc
detection. Upon readjustment of the relative
concentrations to enhance the production of CCC, the
presence of CCC could now be detected. Following the

20   procedure described for modifying the concentrations with
resorcinol as the core compound, the cube root of
[AAA]/[BBB] was taken to obtain the factor by which the
current concentration of B needs to be multiplied such
that [AAA] = [BBB], e.g. 1.2 x 15 mmol = 18 mmol; (ii) by

25   obtaining the value of [BCB]/[BCC], which yields the
factor by which the current concentration of C must be
multiplied such that [BCB] = [BCC], and [BBB] =[AAA], e.g.
1.7 x 1.2 x 36 mmol = 73 mmol. The yields of the resulting

31

ten tri-O-alkylated products showed an improved R = 11, as compared to the previous R = 12.2. The results are reported for the first and second studies in Tables 4 and 5, respectively.

5   Table 3

Product	M+ yield (Area x 10^6)	M+% of total	Total ion yield	M.W. (amu)	Rel. molor yield	Rel. mol. yield, normalized to AAA
AAA	0.114	0.050	2.278	252	0.009	1.00
BAA	0.490	0.051	9.524	324	0.029	3.22
CAA	0.357	0.041	8.686	330	0.026	2.89
ABB	0.673	0.049	13.87	396	0.035	3.89
ABC	0.904	0.037	24.40	402	0.061	6.78
ACC	0.207	0.019	10.68	408	0.026	2.89
BBB	0.061	0.026	2.302	468	0.005	0.56
BBC	0.113	0.014	8.316	474	0.017	1.89
BCC	0.041	0.008	5.102	480	0.011	1.22

[ABC]/[BBB] = 12.2

Product	Mathematical fitting expression
AAA	$[A]^3 k_A^3$
20 BAA	$3[A]^2[B] k_A^2 k_B$
CAA	$3[A]^2[C] k_A^2 k_C$
ABB	$3[A][B]^2 k_A k_B^2$
ABC	$6[A][B][C] k_A k_B k_C$

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	ACC	$3[A][C]^2 k_A k_C^2$
	BBB	$[B]^3 k_B^3$
	BBC	$3[B]^2[C] k_B^2 k_C$
	BCC	$3[B][C]^2 k_B k_C^2$
5	CCC	$[C]^3 k_C^3$

Table 4

Product	M+ yield (Area x 10⁻⁶)	M+% of total	Total ion yield	M.W. (amu)	Rel. molar yield	Rel. mol. yield, normalized to AAA
AAA	0.075	0.049	1.53	252	0.006	1.00
BAA	0.335	0.046	7.28	324	0.023	3.83
CAA	0.373	0.042	8.88	330	0.027	4.50
ABB	0.395	0.047	8.40	396	0.021	3.50
ABC	0.986	0.039	25.3	402	0.063	10.5
ACC	0.453	0.025	18.1	408	0.044	7.33
BBB	0.041	0.017	2.43	468	0.005	0.90
BBC	0.168	0.015	11.2	474	0.024	4.00
BCC	0.170	0.012	14.2	480	0.030	5.00
CCC	0.022	0.005	4.40	486	0.009	1.52

EXAMPLE 3

Mixed O-Alkylations of Resorcinol

The adduct reagents were primary alkyl bromides:
bromopropane (A); 3-acetoxypropyl-1 bromide (B);
phenoxyethyl bromide (C); D-citronellyl bromide (D); and
3-cyanopropyl-1 bromide (E). The reaction was carried out
as described in Exaple 1. Mixed derivitizations were
performed, by combining resorcinol pairing each of the
alkyl bromides with A to determine the relative reactivity
under the reaction conditions for each of the alkyl
bromides normalized to the reactivity of A. The weight
ratios of the reactants in the reaction mixture were:

Alkyl Bromide	Relative Reactivity	Amount used mmol
A	1	10
B	1.5	6.7
C	0.8	12.5
D	0.56	17.7
E	3.7	2.7

Using the rate-adjusted quantities of the alkyl bromides
with 1 mmol of resorcinol, the reaction was carried out
for 24h at a temperature of 20°C to produce the expected
15 products. The following table indicates the results.
The presence of the products and their amounts were
determined by a total ion chromatography and mass
spectroscopy. Two of the products, BD and CE, had
identical retention times on the total ion chromatogram
and formed a single large peak at 13.73 min. Single ion
monitoring was used to determine the individual yields of
BD and CE. As seen in the table, the relative yield of
the 15 products fell within a 3.63 range. This is not far

from the ideal range of 2. No significant amount of

products from side reactions were observed in the analyses

of the reaction mixture.

Product (MW,amu)	Retention time (min.)	M⁺ yield (Area/10⁶)	M⁺, % of total	Total M⁺ yield	Relative molar yield	Rel. mol. yield, normalized to AA
AA (194)	8.30	0.284	0.111	2.56	0.013	1.00
BB (338)	13.23	0.321	0.054	5.91	0.017	1.34
CC (350)	14.99	0.323	0.072	4.48	0.013	1.00
DD (386)	14.18	0.182	0.022	8.10	0.021	1.61
EE (244)	12.38	0.579	0.104	5.55	0.023	1.75
AB (266)	11.05	0.512	0.071	7.21	0.027	2.09
AC (272)	12.06	0.554	0.087	6.41	0.024	1.81
AD (290)	11.66	0.329	0.035	9.48	0.033	2.51
AE (219)	10.43	0.621	0.087	7.14	0.033	2.51
BC (344)	14.12	0.879	0.082	10.72	0.031	2.38
BD (362)	13.73	0.475	0.037	12.88	0.036	2.74
BE (291)	12.80	0.751	0.061	12.32	0.042	3.26
CD (368)	14.58	0.464	0.039	11.76	0.032	2.46
CE (297)	13.73	0.943	0.073	12.88	0.043	3.34
DE (315)	13.32	0.304	0.021	14.85	0.047	3.63

Example 4

Determination of rate normalization constants for 10 R'Br
with respect of phloroglucinol

       The conditions for the normalization were 1 mmol

5   phloroglucinol, 15 mmol of KF/Al_2O_3 15 mmol of propyl

bromide and 15 mmol of the test bromide in DMF at 25°C.

The following table indicates the results of the rate

normalization of 10 aliphatic bromides.

	Test Bromide	Normalization factor
A	propyl	1
B	cyclopropylmethyl	2
C	oxiranylmethyl	3
D	2-methoxyethyl	2
E	3-cyanopropyl	0.23 (0.27*)
F	3-methylbutyl	1.67
G	hexyl	0.8
H	2-methoxyethoxy-ethylene	3
I	4-acetoxybutyl	0.67

10   B

15   G

J   citronellyl   1.60 (1.84*)

                                                                    * with respect to

                                                                    resorcinol

II. Reaction of Phloroglucinol with Primary Set of 10 Rate Adjusted R'Br

v + 3, n = 10

5   Total number of compounds possible is 220.

R'Br	mL	mmol
A	0.43	4.71
B	0.91	9.42
C	1.21	14.13
D	0.89	9.42
E	0.12	1.10
F	0.94	7.85
G	0.53	0.43
H	1.92	14.13
I	0.454	3.14
J	1.49	7.54
Total	8.88	75.2

Using the GC-MS analysis described previously,
molecular ions were detected for all of the possible
products except for DDD, EEE, JJJ, and DEJ, where the
peaks may have been obscured by a stronger peak from
5   another compound.   In other less complex preparations,
these compounds were observed.   The most abundant products
were BGI (17.89 min), DHJ (19.13 mn), FGI (17.78 min),
and FHJ (19.26 min), EFI and FHI (18.50 min), BEI and FGJ
(18.60 min), ABJ and AEH (16.73 min).
10   The experimental ratio of BGI/GGG was 17, where the
ideal equimolar yield predicts a ratio of 6.   The
deviation from the ideal was 2.8

It is evident from the above results, that one can
produce fairly sophisticated libraries very quickly and
15   easily, which allow for screening of the resulting
mixtures.   Where a mixture is found to be active, if the
mixture is too complex to be analyzed directly, a second
round of reactions is carried out, so that ultimately one
can define the compound(s) present in the mixture which
20   provides the activity.   The method allows for using
reactions in solution, so as to avoid the various problems
associated with reactions on surfaces.   In addition, it
allows for great versatility and type of compounds which
can be produced, so as to provide for high probabilities
25   of defining useful compounds associated with a particular
application.

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All publications and patent applications cited in this

specification are herein incorporated by reference as if

each individual publication or patent application were

specifically and individually indicated to be incorporated

5   by reference.

    Although the foregoing invention has been described

in some detail by way of illustration and example for

purposes of clarity of understanding, it will be readily

apparent to those of ordinary skill in the art in light of

10 the teachings of this invention that certain changes and

modifications may be made thereto without departing from

the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

    1.   A method for preparing a large number of related compounds in a plurality of complex mixtures, which method allows for the screening of the mixtures as to the

5   identity of compounds having a designated characteristic, said method comprising:

    as a first or intermediate stage, introducing under reaction conditions into each of at least three vessels a common polyfunctionalized core molecule and a first set of

10   adduct molecules which react with said core molecule, where each first set of adduct molecules is characterized by being different from the other first sets, overlapping, having fewer than the total number of adduct molecules and the combined products from each first set includes

15   substantially all possible per-substituted core molecules, to provide at least three first product mixtures;

    screening each of said first product mixtures for a designated activity to provide a first set active mixture;

    as a next stage, introducing under reaction

20   conditions into each of at least three vessels said common polyfunctionalized core molecule and a second set of adduct molecules which react with said core molecule, where each second set is characterized by consisting of members of said first set active mixture, being different

25   from the other second sets, overlapping, having fewer than the total number of adduct molecules of said first set active mixture and the combined products from each second

set includes substantially all possible per-substituted

core molecules, to provide at least three second product

mixtures;

screening each of said second product mixtures for a

5 .designated activity to provide a second set active

mixture; and

optionally repeating said next stage and screening

until at least one compound is characterized as having

said designated activity.

10   2.   A method according to Claim 1, wherein the

relative concentrations of each of the adduct molecules in

each of the sets results in substantially equivalent

concentrations of each of the products.

3.   A method according to Claim 1, wherein said core

15 molecule has from 2 to 5 of the same functional groups and

the number of vessels is at least 5.

4.   A method according to Claim 3, wherein the

number of adduct molecules in each vessel is at least 6 in

the first stage.

20   5.   A method according to Claim 1, wherein said

screening is for binding to a protein.

6.   A method according to Claim 1, wherein each of said adduct molecules is in substantial excess to stoichiometry.

7.   A method for preparing at least 100 related

5   compounds in a plurality of complex mixtures, which method allows for the screening of the mixtures as to the identity of compounds having a designated characteristic, said method comprising:

as a first or intermediate stage, introducing under

10   reaction conditions into each of at least three vessels a common polyfunctionalized core molecule having the same functional groups and a first set of adduct molecules which react with said core molecule, where each first set of adduct molecules is characterized by being derived from

15   a union of subset units, different from the other first sets, overlapping, having fewer than the total number of adduct molecules, the relative concentrations of each of the adduct molecules in each of the sets results in substantially equivalent concentrations of each of the

20   products, and the combined products from each first set includes substantially all possible per-substituted core molecules, to provide at least three first product mixtures, wherein each subset unit is non-overlapping with other subset units, the number of subsets is or greater

25   than the number of functionalities on said core molecule, and wherein each set has an equal number of at least two subsets and fewer than the total number of subsets;

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screening each of said first product mixtures for a

designated activity to provide a first set active mixture;

as a next stage, introducing under reaction

conditions into each of at least three vessels said common

5   polyfunctionalized core molecule having the same funcional

groups and a second set of adduct molecules which react

with said core molecule, where each second set is

characterized by consisting of members of said first set

active mixture, being different from the other second

10   sets, overlapping, having fewer than the total number of

adduct molecules of said first set active mixture and the

combined products from each second set includes

substantially all possible per-substituted core molecules,

to provide at least three second product mixtures;

15   screening each of said second product mixtures for a

designated activity to provide a second set active

mixture; and

optionally repeating said next stage and screening

until at least one compound is characterized as having

20   said designated activity.

8.   A method according to Claim 7, wherein said core

molecule is functionalized with oxy, oxo, amino thiol,

nitrile, isocyanate, isothiocyanate, carbamate,

thiocarbamate or hydrazine groups.

9.   A method according to Claim 8, wherein said core
molecule has an aromatic core and is symmetrical as to the
functional groups.

10.   A method according to Claim 7, wherein said core
5   molecule has from 2 to 5 functional groups and the number
of vessels is at least 6.

11.   A method according to Claim 7, wherein the
number of adduct molecules in each vessel is at least 6 in
the first stage.

10   12.   A method according to Claim 7, wherein said
screening is for binding to a protein.

13.   A method according to Claim 7, wherein each of
said adduct molecules is in substantial excess to
stoichiometry.

15   14.   A method for preparing at least 1000 related
compounds in a plurality of complex mixtures, which method
allows for the screening of the mixtures as to the
identity of compounds having a designated characteristic,
said method comprising:
20   as a first or intermediate stage, introducing under
reaction conditions into each of at least three vessels
containing a common polyfunctionalized core molecule a
first set of adduct molecules which react with said core

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molecule, where each first set of adduct molecules is
characterized by being derived from a union of subset
units, different from the other first sets, overlapping,
having fewer than the total number of at least 12 adduct

5   molecules, the amounts of each of said adduct molecules
being in substantial stoichiometic excess, the relative
concentrations of each of the adduct molecules in each of
the sets results in substantially equivalent
concentrations of each of the products, and the combined

10   products from each first set includes substantially all
possible per-substituted core molecules, to provide at
least four first product mixtures, wherein each subset
unit is non-overlapping with other subset units, the
number of subsets is greater than the number of

15   functionalities on said core molecule, and wherein each
set has an equal number of at least two subsets and fewer
than the total number of subsets;

        screening each of said first product mixtures for a
designated activity to provide a first set active mixture;

20   as a next stage, introducing under reaction
conditions into each of at least three vessels said common
polyfunctionalized core molecule and a second set of
adduct molecules which react with said core molecule,
where each second set is characterized by consisting of

25   members of said first set active mixture, being different
from the other second sets, overlapping, having fewer than
the total number of adduct molecules of said first set
active mixture and the combined products from each second

set includes substantially all possible per-substituted

core molecules, to provide at least three second product

mixtures;

screening each of said second product mixtures for a

5   designated activity to provide a second set active

mixture; and

optionally repeating said next stage and screening

until at least one compound is characterized as having

said designated activity.

10   15.   A method for preparing a large number of related

compounds in a plurality of complex mixtures, which method

allows for the screening of the mixtures as to the

identity of compounds having a designated characteristic,

said method comprising:

15   as a first or intermediate stage, introducing under

reaction conditions into each of at least three vessels a

common polyfunctionalized core molecule bonded by a

cleavable linkage to a solid support and a first set of

adduct molecules which react with said core molecule,

20   where each first set of adduct molecules is characterized

by being different from the other first sets, overlapping,

having fewer than the total number of adduct molecules and

the combined products from each first set includes

substantially all possible per-substituted core molecules,

25   to provide at least three first product mixtures;

screening each of said first product mixtures for a

designated activity to provide a first set active mixture,

while said product mixture is bound to said solid support
or released from said solid support;

as a next stage, introducing under reaction
conditions into each of at least three vessels said common
5   polyfunctionalized core molecule bound to a solid support
and a second set of adduct molecules which react with said
core molecule, where each second set is characterized by
consisting of members of said first set active mixture,
being different from the other second sets, overlapping,
10 having fewer than the total number of adduct molecules of
said first set active mixture and the combined products
from each second set includes substantially all possible
per-substituted core molecules, to provide at least three
second product mixtures;
15 screening each of said second product mixtures for a
designated activity to provide a second set active
mixture, while said product mixture is bound to said solid
support or released from said solid support; and

optionally repeating said next stage and screening
20 until at least one compound is characterized as having
said designated activity.

16.   A method according to Claim 15, wherein said
solid support is a particle.

17.   A method according to Claim 15, wherein said
25 solid support is a vessel wall.

18.   A combinatorial library produced according to
the method of introducing under reaction conditions into
each of at least three vessels a common polyfunctionalized
core molecule and a first set of adduct molecules which
5   react with said core molecule, where each first set of
adduct molecules is characterized by being different from
the other first sets, overlapping, having fewer than the
total number of adduct molecules and the combined products
from each first set includes substantially all possible
10   per-substituted core molecules, to provide at least three
first product mixtures.

19.   A combinatorial library produced according to
the method of introducing under reaction conditions into
each of at least three vessels a common polyfunctionalized
15   core molecule having the same functional groups and a
first set of adduct molecules which react with said core
molecule, where each first set of adduct molecules is
characterized by being derived from a union of subset
units, different from the other first sets, overlapping,
20   having fewer than the total number of adduct molecules,
the relative concentrations of each of the adduct
molecules in each of the sets results in substantially
equivalent concentrations of each of the products, and the
combined products from each first set include
25   substantially all possible per-substituted core molecules,
to provide at least three first product mixtures, wherein
each subset unit is non-overlapping with other subset

units, the number of subsets is greater than the number of
functionalities on said core molecule, and wherein each
set has an equal number of at least two subsets and fewer
than the total number of subsets.

5   20.   A combinatorial library according to Claim 19,
wherein said core molecule is functionalized with oxy,
oxo, amino thiol, nitrile, isocyanate, isothiocyanate,
carbamate, thiocarbamate or hydrazine groups.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :G01N 33/52; C12Q 1/00
US CL   :435/7.1; 436/501, 518, 35, 36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1; 436/501, 518, 35, 36

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, APS

search terms: structure search, combinatorial, library, array

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 86/00991 (GEYSEN) 13 FEBRUARY 1986, PAGE 4, LINE 16-PAGE 6, LINE 4	1-20
Y	WO, A, 91/18598 (LIU ET AL.) 12 DECEMBER 1991, SEE PAGE 5.	1-20
Y,P	JOURNAL OF MEDICINAL CHEMISTRY, VOLUME 37, NUMBER 10, ISSUED 13 MAY 1994, GORDON ET AL., "APPLICATIONS OF COMBINATORIAL TECHNOLOGIES TO DRUG DISCOVERY. 2. COMBINATORIAL ORGANIC SYNTHESIS, LIBRARY SCREENING STRATEGIES, AND FUTURE DIRECTIONS", PAGES 1385-1401, SEE ENTIRE ARTICLE.	1-20

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 JANUARY 1995	13 FEB 1995

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Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ACCOUNTS OF CHEMICAL RESEARCH, VOLUME 11, ISSUED 1978, LEZNOFF, "THE USE OF INSOLUBLE POLYMER SUPPORTS IN GENERAL ORGANIC SYNTHESIS", PAGES 327-333, SEE PAGE 327.	12-15